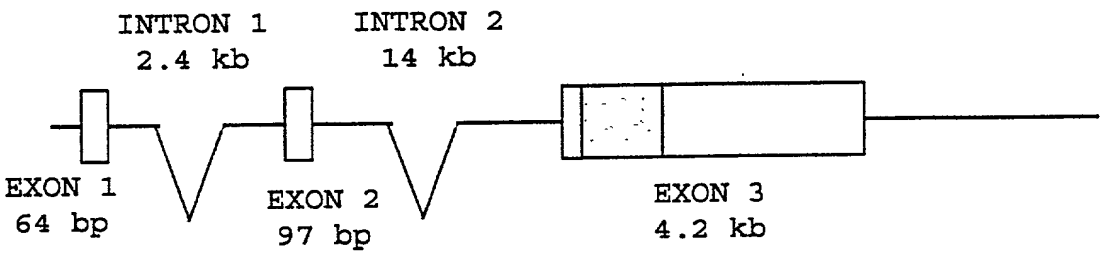


FIG. 1

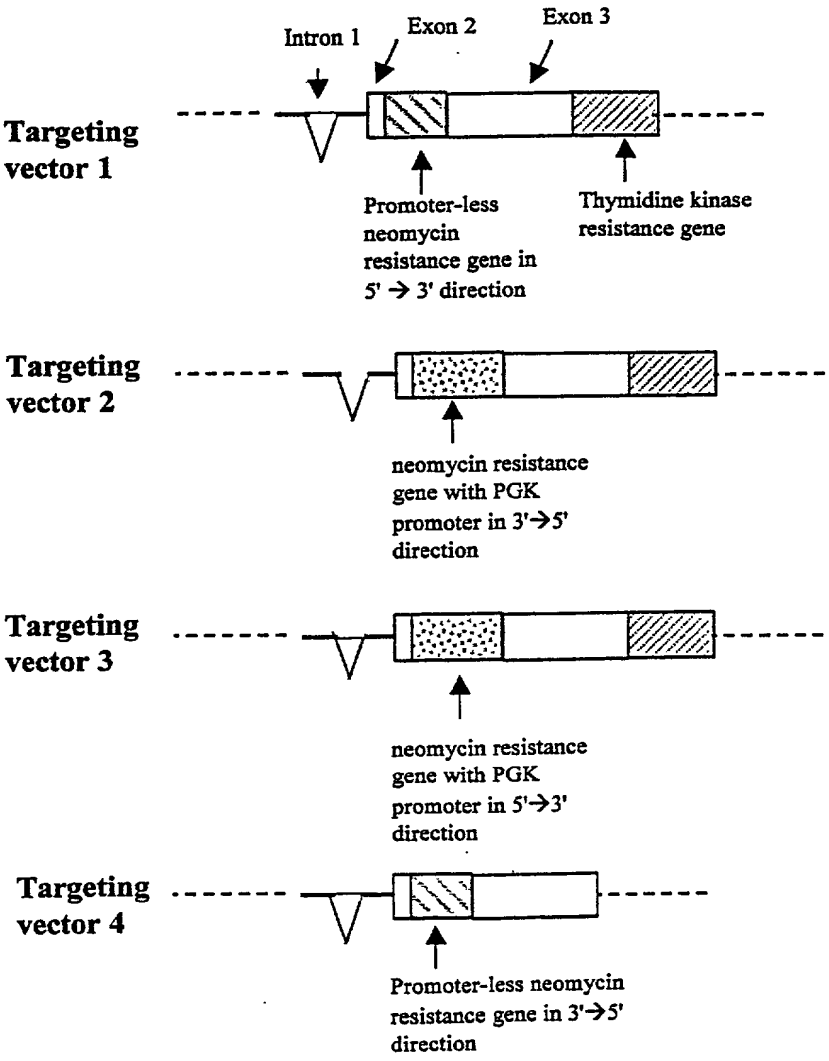

Intron 1
Exon 2
Exon 3
Targeting vector 1
Promoter-less neomycin resistance gene in 5' → 3' direction
Thymidine kinase resistance gene
Targeting vector 2
neomycin resistance gene with PGK promoter in 3'→5' direction
Targeting vector 3
neomycin resistance gene with PGK promoter in 5'→3' direction
Targeting vector 4
Promoter-less neomycin resistance gene in 3'→5' direction

FIG. 2A

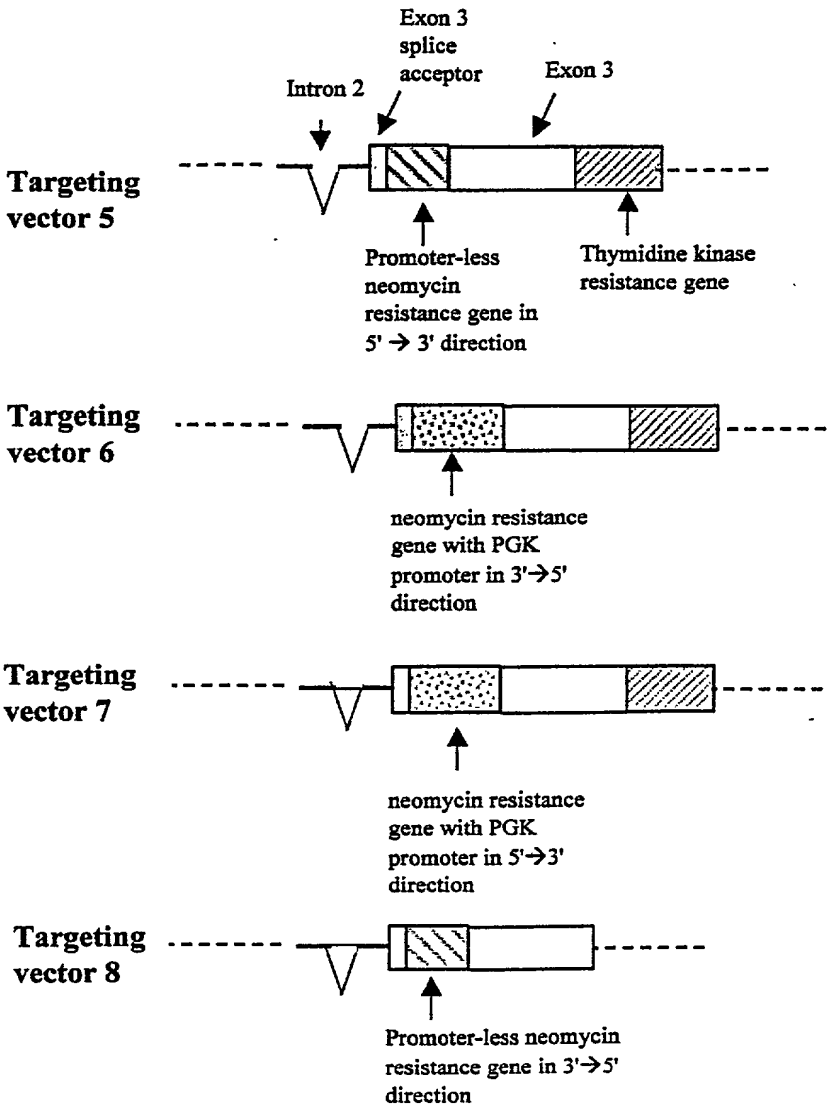

Exon 3
splice
acceptor
Intron 2
Exon 3
Targeting
vector 5
Promoter-less
neomycin
resistance gene in
5' → 3' direction
Thymidine kinase
resistance gene
Targeting
vector 6
neomycin resistance
gene with PGK
promoter in 3'→5'
direction
Targeting
vector 7
neomycin resistance
gene with PGK
promoter in 5'→3'
direction
Targeting
vector 8
Promoter-less neomycin
resistance gene in 3'→5'
direction

FIG. 2B

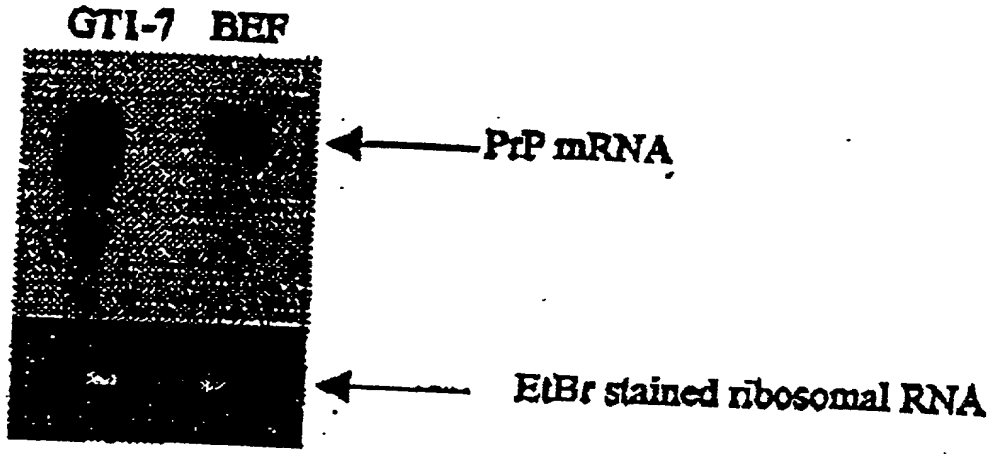


FIG. 3

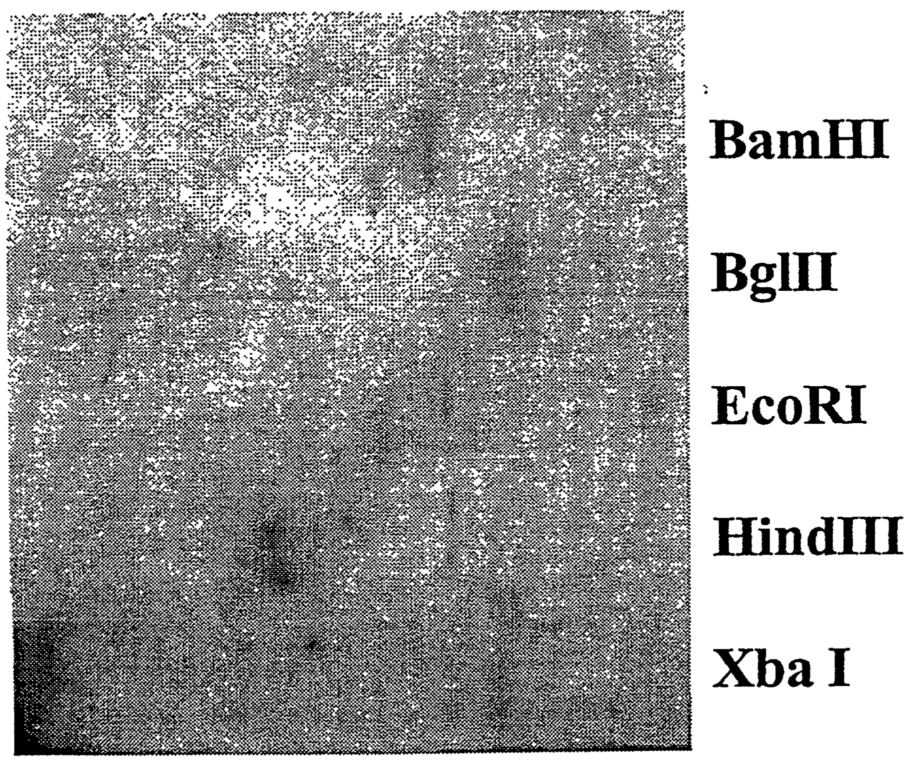


FIG. 4

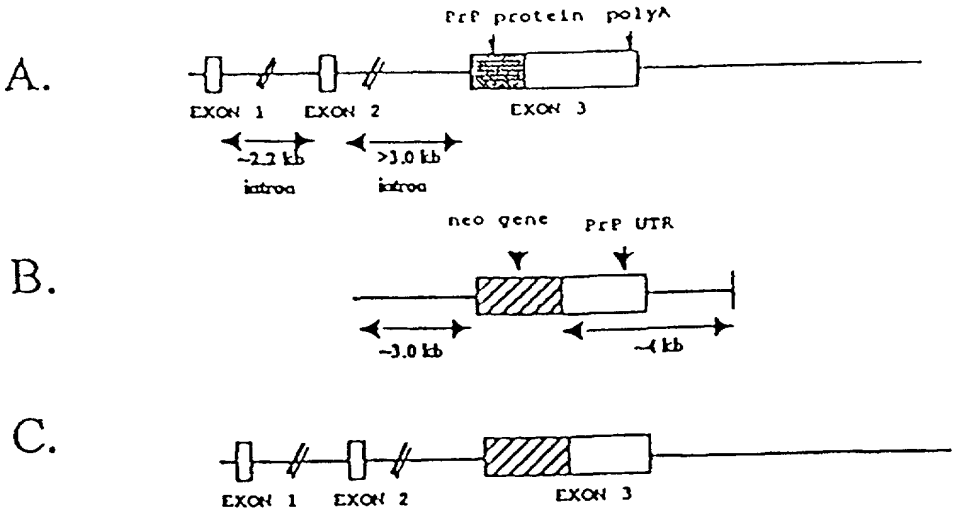

A.
PrP protein
polyA
EXON 1
EXON 2
EXON 3
~2.2 kb
intron
>3.0 kb
intron
B.
neo gene
PrP UTR
~3.0 kb
~1 kb
C.
EXON 1
EXON 2
EXON 3

Figure 5

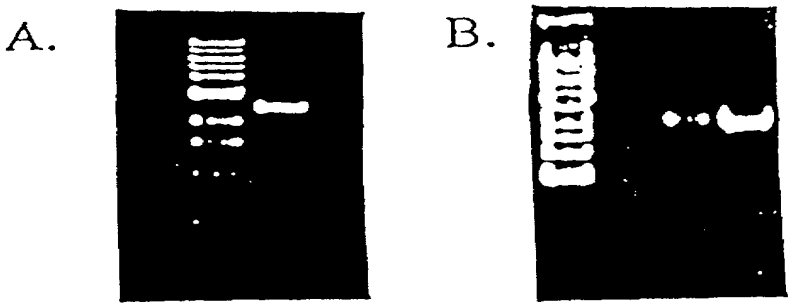


Figure 6

Figure 7 Cell survival in electroporation of BFF transfected with pPNT and pPRP.

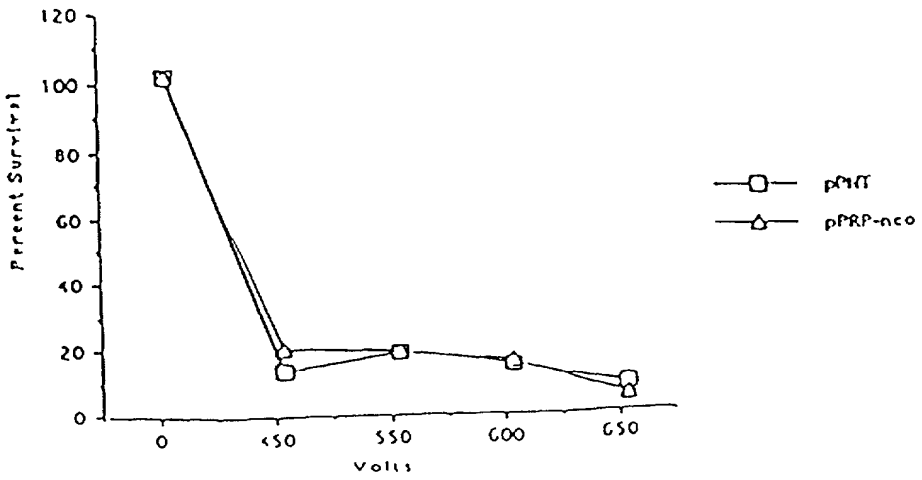


Figure 8 Electroporation of BFF transfected with pPNT - second experiment

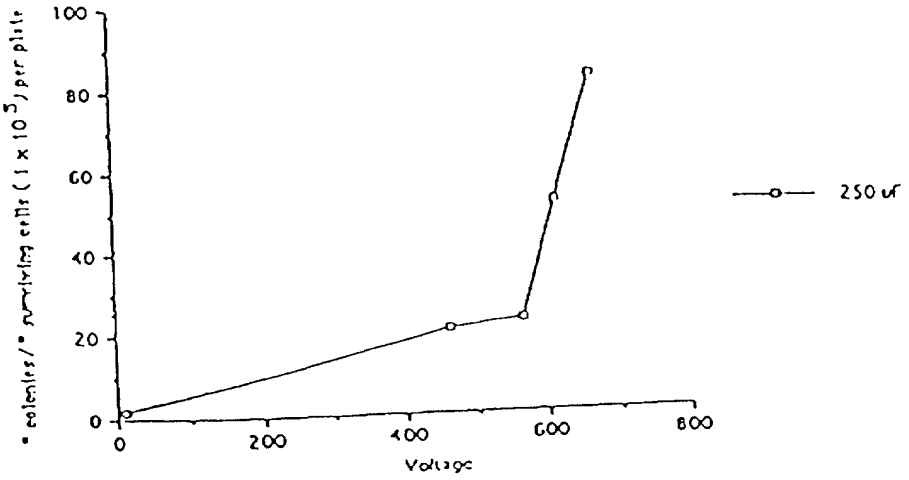


Figure 9 Electroporation of BFF transfected with pPRP.

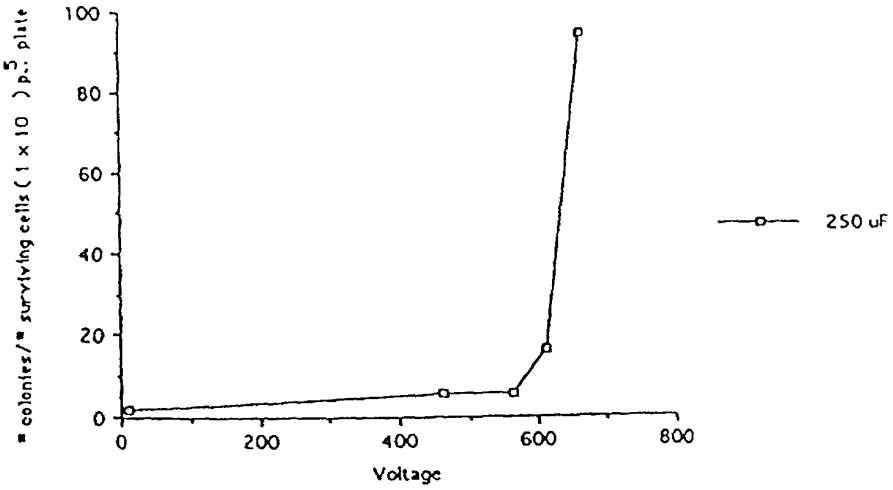


Figure 10 G418 Treatment of untransfected BFF cells.

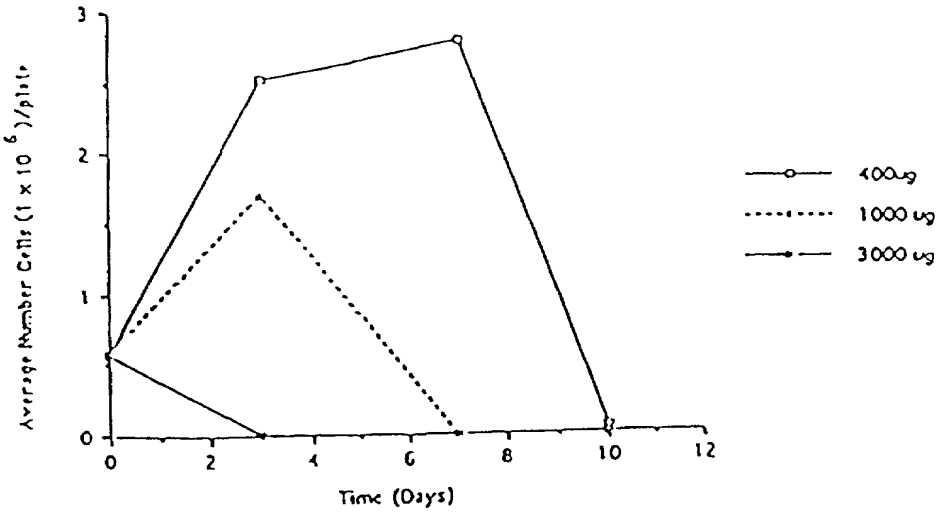


Figure 11

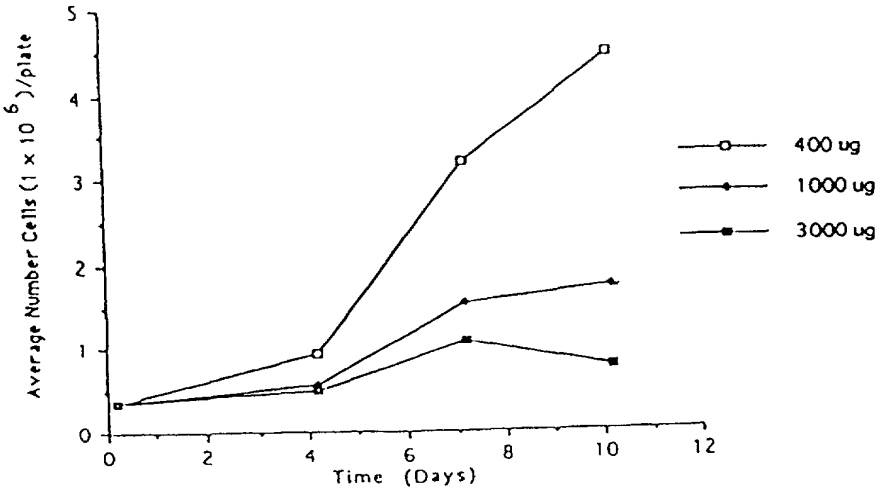
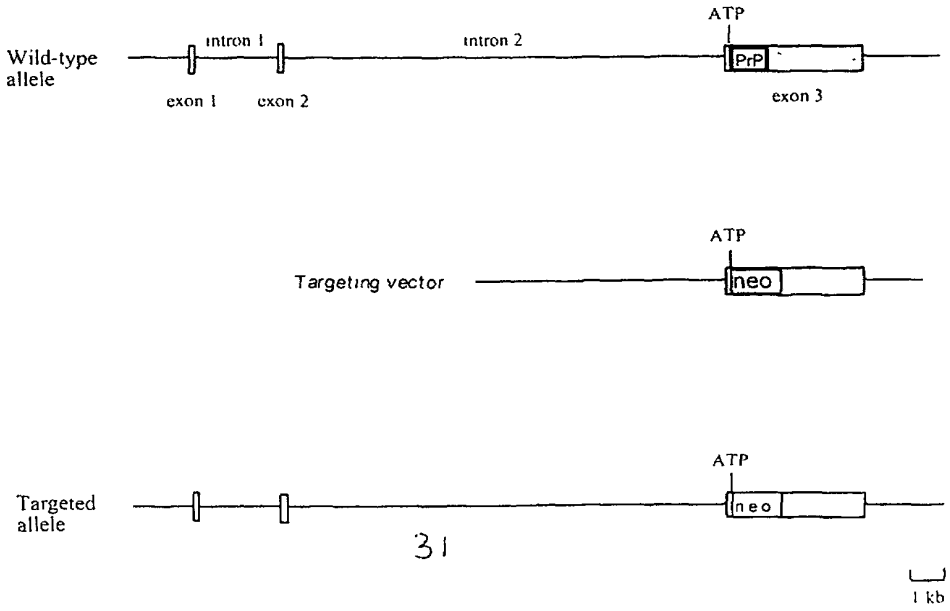


Figure 12. Genomic DNA organization of bovine PrP and the gene targeting strategy.



Top: The bovine PrP gene is composed of 3 exons and two introns, spanning an over 20 kb region [43]. Exon I and 2, which are 53 by and 98 by in size respectively, are transcribed as S' UTR, and the third exon contains sequences of 10 by S' UTR, 795 by coding region and about 3.3 kb 3' UTR. Intron 1 and 2 are about 2.4 kb and 14 kb in size.

Middle: The gene targeting vector contains a part of intron 2 sequence (at least 7 kb, exon 3 in which the bovine PrP coding sequence is completely deleted and replaced with promoterless neomycin resistant gene, and partial downstream genomic sequence of exon 3. The expression of neomycin resistant gene is under the control of the endogenous PrP promoter and its regulatory elements.

Bottom: The targeted bovine PrP allele after homologous recombination. Shaded boxes, exons; open boxes with names of gene, coding sequence the genes. ATP, start codon.